VI.—The Gonadotrope Actions of the Anterior Lobe of the Pituitary.

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1. The Diphasic Nature of the Sex Cycle of the Rat and Mouse.

The problem of the relation between gonad and the other endocrine glands has, during recent years, attracted much attention. It has been shown that the maturation of the ovary, which is responsible for the other manifestations of puberty, is itself dependent upon the developmental level of its somatic environment rather than upon its own age; that the senescent insufficiency of the ovary is due, not so much to its own inability to elaborate hormones and ova, as to a decrease in the amount of, or deterioration in the quality of, the extra-ovarian substances which are necessary for its proper functioning. Similar evidence has been produced in relation to the testis. It has been shown, further, that the pituitary is intimately concerned in the establishment and maintenance of the œstrous cycle, and also that the placenta exerts an influence upon the structure of the ovary.

Much of this newer knowledge has been gained by a study of the influence of the pituitary upon the œstrous cycle of the mouse and rat. A considerable body of facts has emerged from these studies, but, without doubt, "the evidence so far produced is not only inconclusive but even contradictory" (Bellerby). For example, absence of œstrus was noted in hypophysectomised rats; the ovaries were found to be atrophic. This atrophy can be repaired and the œstrous cycle induced in hypophysectomised animals by grafts of anterior lobe substance. Moreover, the number of eggs shed during ovulation in the normal mature animal can be increased, and the prepuberal ovary of the immature animal can be activated by such grafts (Smith and Engle, Zondek and Aschheim). On the other hand, it has been reported by Evans that alkaline extracts of the anterior lobe, which encourage growth, inhibit the œstrous cycle in mature animals. The contrast between these *œstrogenic grafts* and the *œstrus-inhibiting extracts* is accentuated by the fact that the latter do not merely prevent œstrus in the normal animal, but actually inhibit and neutralise the œstrogenic action of grafts.

Recently various methods of producing œstrogenic extracts have been described by independent workers—Zondek and Aschheim, Evans and Simpson, Wiesner and Crew—but the fact that certain extracts of an endocrine gland apparently oppose and inhibit the typical action of grafts of the same gland still remains to be explained. The conclusion of Putnam and his colleagues that these œstrus-inhibiting extracts can also induce œstrus in immature animals, and thus produce the effect of a graft, has not been confirmed (Bellerby).

A further complication has been created by the demonstration of the presence of a growth-hormone in some extracts of the pituitary. Evans and Simpson suggest that the œstrus-inhibiting extracts represent a mixture of this growth-hormone and of the sex-hormone, and that the former exerts an antagonism to the latter, and thus embarrasses its action. But it has been reported by Laqueur that substances which are present in the urine of pregnant women and which exert no growth-promoting action can also inhibit the œstrus periodicity. Further, the assumed antagonism between sex-hormone and growth-hormone cannot account for certain attributes of the extracts, for these are reported to possess the property of prolonging the duration of pregnancy, and to exert other intensive activities which represent much more than the mere negation and nullification of the action of the "sex-hormone" of the anterior lobe by its growth-hormone (Teel, Parkes).

The substances from the urine of pregnant women which exert an œstrus-inhibiting effect under certain circumstances are, under other circumstances, actually œstrogenic (Zondek and Aschheim); but while these authors do not hesitate to apply the term "anterior lobe hormone" to these substances, they comment upon their varying effects upon the œstrous periodicity. Because of this varying effect they choose, as a test for the presence of these substances in any given extract or substance, the production of certain structural changes in the ovary, *e.g.* hæmorrhagic follicles, and not the inhibition or the induction of the œstrous cycle and ovulation, which are the primary actions of anterior lobe grafts and extracts. But they conclude that the anterior lobe secretes "a" hormone which is responsible for the functioning of the gonad, inciting ovulation and the production of the ovarian hormone. This hormone of the hypophysis is, in the literature of the subject, commonly referred to as "the sex-hormone of the anterior lobe," or even as "the anterior lobe hormone" (Zondek and Aschheim), in spite of the fact that the existence of an additional growth-promoting hormone has been demonstrated, and that all such assumptions do not account for the various results.

It is perhaps not impossible to reconcile the contradictory facts which thus present themselves and to explain them by various hypothesis; but rather than construct such reconciling assumptions it was thought best to approach the matter from a different angle, since certain facts of ovarian physiology have not, or not sufficiently, been considered by previous authors. A consideration of these newer facts leads to a different formulation of the problem of the influences of the anterior lobe upon the ovary, and to the application of methods somewhat different from those employed heretofore. These facts refer to the *phasic* nature of ovarian secretion, and to the correlation between, and the co-ordination of, structural changes in the ovary and the phases of its endocrine activity.

Most of the work upon this subject, it will be noted, has been carried out upon mice and rats, and the œstrous cycle of these animals is regarded as the criterion of normal ovarian activity. Thus it is that, in almost all papers on this subject, reference is made to the œstrous cycle only when the ovarian endocrine function is being discussed, and the term œstrous cycle has come to be used as a synonym for the complete sex cycle. But it has been shown that in these forms the sex cycle is definitely diphasic and dicyclic, and that the phases are quite distinct (Wiesner, 1926). It is now known that the genital organs *first* pass through a series of changes which together constitute the œstrous cycle, and that at the end of this they return to the stage of rest (interval). During this cycle the mating reflex can be incited in the female. If mating does not occur, another œstrous cycle follows after an interval of varying duration; but if mating occurs, or if the stimulation exerted by the vaginal plug is provoked artificially, a *second* series of changes begins, and during this the uterus acquires the faculty of forming decidual tissue, whilst the vaginal epithelium develops a layer of mucous cells. If fertilisation has occurred, then the potencies of the uterus are utilised and pregnancy results. In the absence of fertilisation pseudo-pregnancy ensues, for the reason that these potencies are developed but are not utilised (Evans and Long, Wiesner). There are thus (in addition to the phase of inactivity, rest or *interval*, usually referred to as diœstrum) two distinct phases of genital activity—the sex or *œstrous phase* and the *reproductive phase*. It is manifest that the absence of œstrus can be due either to the onset of the reproductive phase of genital activity or else to the onset of the stage of rest. (It is suggested that the term "interval" is more appropriate for the latter than is diœstrum, and therefore it will be used in this discussion.)

The genital cycle thus consists of the sequence of œstrous cycle and reproductive cycle, beginning and ending in interval.

2. The Diphasic Nature of Ovarian Secretion.

It has been shown by numerous investigators that certain of the changes associated with the œstrous cycle (*e.g.* cornification of the vaginal epithelium) can be produced by the administration of extracts from the ovary and other organs, such as those prepared by Allen and Doisy. It is more than doubtful, however, whether *all* the changes, normally occurring during the œstrous phase, can be incited by this Allen-Doisy factor. It does not seem to incite in the ovariectomised mouse the rise in metabolism which occurs after cornification (Fraser and Wiesner). Thus the name "œstrin," which has commonly been used in the literature on the subject, seems to be as yet inappropriate, and the less committal name "*alpha factor*" has been proposed and will be used in its place. The secretion of œstrous hormone (of which alpha is one factor) does not represent, however, the only endocrine function of the ovary. For it has been shown by numerous experiments that the second or reproductive phase of the sex cycle is also dependent on the ovarian function. The interruption of pseudo-pregnancy following ovariectomy, the occurrence of pseudo-pregnancy in animals with auto-transplanted ovaries, and similar experiments, have indicated that these changes are dependent on ovarian secretion (Bouin and Ancel, Marshall and Hammond, Evans, *et al.*). On the other hand, it has been shown that injections of alpha factor do not produce the changes typical of the second phase of the sex cycle, and indeed even interrupt these when they are already developing in the mature female (Wiesner, Kunze and Loeb, Corner, Benoit). Moreover, a substance has been extracted from the corpus luteum and from the blood of pregnant animals which does not incite the œstrous changes, but which actually produces certain of the phenomena characteristic of, and regularly occurring in, the reproductive phase of the sex cycle (Hisaw, Wiesner and Patel, Corner and Allen). These observations, together with others secured from experimentation involving the ablation of the corpus luteum (*cf.* Marshall), lead to the conclusion that the reproductive phase of the sex cycle is produced by the action of a hormone which is elaborated in the corpus luteum. In fact, it has been referred to as the "corpus luteum hormone" by Hisaw and by Corner and Allen, and as the "*beta factor*" by ourselves, for the reason that it seems to us that until it has been shown that the corpus luteum

contains no other autacoid, and that this particular hormone is to be found only in the corpus luteum, it is undesirable to suggest any such exclusive specificity.

Experimentation has shown that in all probability the interval is due to transient ovarian inactivity. It would seem that in the unmated female phases of alpha secretion occur at regular intervals, being separated one from the other by phases of endocrine inactivity; this alteration accounting for the periodic incidence of œstrus (Wiesner, 1926). Thus three different phases in the endocrine behaviour of the ovary are to be distinguished—the first phase (alpha production), the second phase (beta production), and the phase of endocrine inactivity, and that these are associated respectively with the three phases of the genital behaviour—œstrous cycle, reproductive cycle, and interval.

The phasic nature of ovarian activity must be considered when the problem of the relation between pituitary and gonad is being discussed. It is necessary to inquire as to the relations between the different phases of endocrine ovarian activity and the endocrine activity of the pituitary. Further, the problem of the inhibition of œstrus must now be considered in the light of the existence of the three ovarian phases. The occurrence of the œstrous cycle (in the genital organs) can be due only to the secretion of alpha factor by the ovary, *i.e.* to the appearance of an alpha phase; but the absence of œstrus can be a reflection either of the phase of endocrine inactivity, or else of a production of the beta hormone, *i.e.* to the establishment of the beta phase.

In the normal mature animal both of these causes are actually responsible for the periodic abeyance of œstrus. Œstrus is never observed in the pregnant or pseudo-pregnant animal, *i.e.* when a beta phase prevails; on the other hand, the absence of the œstrous changes between the stages of heat in the unmated female is due to the inactivity of the ovary. It is seen, therefore, that the mere absence of œstrus or interruption of œstrous periodicity does not in itself warrant any definite conclusion being made as to its actual cause, since it may be due either to a transient ovarian anhormony or else to a stage of high secretory activity.

The bearing of this upon the question of the œstrus-inhibiting extracts from the anterior lobe is manifest. It has to be decided whether such extracts are merely antagonistic to any ovarian secretion, or whether they invoke the development of the beta phase. It is of interest to note, in this context, that maturation of the immature ovary, with the formation of mature follicles and the secretion of alpha hormone, which occurs after the implantation of anterior lobe substance, has been described as a

process suppressed by the action of Evans' alkaline extracts, which induce the formation of corpora lutea without the elaboration of alpha hormone. But although maturity normally *begins* with an alpha phase (Allen, Evans and Long, Wiesner, Zondek and Aschheim, Steinach, *et al.*), it has to be remembered that maturity of the ovary can refer to either of the two phases of endocrine activity; the non-occurrence of œstrus does not necessarily mean that the ovary is immature, since it may have entered the beta phase. It is not permissible, therefore, to reject the term "mature" merely because the ovary has not passed into the alpha phase.

It therefore appears that the problem of the influence of the anterior lobe upon the endocrine function of the ovary is a problem of its influence upon the phases of ovarian endocrine activity. In any analysis of pituitary effect it becomes necessary to identify the actual functional stage of the ovary in order to establish whether the phase of rest, the alpha phase, or the beta phase prevails at any given moment.

3. The Identification of the Different Phases of Ovarian Endocrine Activity by an Appeal to Structural Peculiarities.

(A) *The Alpha Phase.*

Thus one has to devise methods for the identification of the functional condition of the ovary at any given time.

It is well known that during the œstrous cycle follicular development begins with, and culminates in, ovulation, whereas the reproductive phase of the cycle is usually associated with a persistence of corpora lutea. Some authorities, indeed, speak of a follicular and of a luteal phase of the ovarian cycle, referring only to the structural changes, but assigning to each of these a definite physiological rôle (Loeb, Fränkel, *et al.*). If such assumptions were justified, then it would be correct to regard the morphological features of the ovary as the true criteria of ovarian endocrine activity. It seems, however, that such conclusions cannot be drawn.

It is true that the œstrous phase of the sex cycle is usually accompanied by maturation of the follicles. Thus the final increase in follicular size which eventually leads to ovulation might be regarded as the indication of the presence and action of alpha hormone. But this conclusion, limited as it is, cannot be made with sufficient safety, for it is based on the tacit assumption that enlargement (maturation of follicles) cannot occur without synchronous alpha production—an assumption not sufficiently well supported by known facts.

While it is not certain that the enlargement of the follicles can be

regarded as a proof of alpha secretion, the absence of follicular enlargement cannot be accepted as a trustworthy indication of endocrine inactivity. During the normal œstrous cycle in the mature rat and mouse, alpha is already secreted at a time when follicular changes are not apparent. This is borne out by the observations of Long and Evans, who have shown that the disappearance of leucocytes in the first stage of the œstrous cycle occurs before any definite follicular enlargement (beyond the maximum interval diameter) is apparent. It is also established that alpha can be secreted in the entire absence of any follicles. Steinach, for example, has shown that the ovarian secretion is not inhibited by the complete destruction of all follicles by means of X-rays. Parkes and Schubert have confirmed his conclusion, and have shown that not even the periodicity of alpha secretion, which is responsible for the periodicity of the œstrous cycle, is necessarily broken following such irradiation. In other experiments it has been shown by Wiesner that the inhibition of follicular maturation by extirpation of the uterus in the rat does not prevent the occurrence of œstrus. Such experimentation led to the conclusion that follicular development and alpha secretion must be mutually independent and incited by a common cause, some hypothetical factor, which then was referred to as "x."

Nor can the presence of corpora lutea be regarded as the criterion of alpha secretion, for in the rat and the mouse the corpora lutea of any ovulation can persist during several of the subsequent cycles without interfering in any way with the secretion of alpha or with ovulation (Long and Evans, Allen). Evans has shown that, after the succeeding œstrous cycle has begun, certain changes occur in the cells of the lutein bodies of the previous ovulation which he thinks to be of a degenerative nature. However, it must be concluded from the changes in the vaginal epithelium preceding and accompanying these supposedly degenerative changes in the corpora lutea that the latter do not occur until about two days after alpha secretion has already begun. Therefore, even though they were more easily identifiable, they could not be regarded as a characteristic sign of alpha secretion. It has been shown, moreover, that the persistence of the corpora lutea after hysterectomy in the rat does not prevent œstrus (Wiesner). Thus the presence of corpora lutea cannot be regarded as an indication of the absence of alpha secretion.

On the other hand, the presence of corpora lutea is not necessary for the occurrence of alpha secretion (Marshall, Allen, *et al.*). Thus it would seem to be impossible to identify the existence of the alpha phase of ovarian endocrine activity from an examination of structural features of

the ovary, though the presence of maturing follicles can certainly be regarded as being suggestive.

(B) *The Beta Phase.*

Several investigators have endeavoured to distinguish a follicular and a lutein phase of the ovarian cycle. At first sight it would seem possible and easy to identify these two suggested phases with the alpha and the beta phase; the presence of corpora lutea being regarded as an indication of the existence of the beta phase, and absence of corpora lutea as an indication of the absence of beta production. Certain facts, however, prevent any such conclusion being drawn. Corpora lutea are formed after every ovulation, but in spite of this, no beta hormone is produced in the rat and the mouse unless there is, in addition, the stimulus exerted by the vaginal plug.* And though it is not improbable that there is to be found a definite structural difference between corpora lutea which are associated with beta production, and others that are not, such differences have not yet been described. The mere presence of corpora lutea cannot serve, therefore, as an indication of beta production. On the other hand, in the normal animal beta production is always associated with the presence of corpora lutea; for it occurs only after mating—*i.e.* after an œstrous cycle during which corpora lutea form. Moreover, beta hormone has so far been found mainly in the corpus luteum, and has not been extracted from other ovarian tissues (Allen and Corner). It would seem reasonable, therefore, to assume that in the absence of any lutein tissue there is no secretion of the beta hormone. Since, however, such a conclusion would be based entirely upon negative findings, being subject to all the inherent fallacies of such conclusions, it was decided to leave open the decision as to whether or not beta secretion can be excluded in the absence of corpora lutea. Experiments which are reported herein have shown that this cautious attitude was justified. Thus the diagnosis of the beta phase, whether it be positive or negative, is not possible on purely morphological grounds.

(C) *The Interval Stage.*

There remains the question as to whether or not it is possible to identify the stage of endocrine rest by an appeal to the morphological structure of the ovary.

* This is manifest from the inability of the uterus to form decidua (Long and Evans), and the absence of mucification in the vaginal epithelium (Wiesner and Patel).

It has been shown that about forty-eight hours elapse before the effect of menformon (a water-soluble substance containing alpha) (Laqueur) is reflected in the vaginal epithelium. It can be concluded from this that the same effect—cornification—in the normal spontaneous cycle of the mature mouse is due to the secretion of alpha occurring about two days before these morphological changes present themselves. Ablation experiments lead to a similar conclusion (Parkes). But at the time when, according to this interpretation, alpha secretion occurs, the ovary actually is in a "resting" condition as regards its structural conditions, no enlarged follicles being present. This condition cannot serve, therefore, as an indication of endocrine inactivity.

It has been noted that the presence of corpora lutea can coincide both with the alpha and the beta phase, and it must be remembered that corpora lutea are also present in the ovary between the œstrous cycles, when no secretion of hormone takes place. Thus the stage of ovarian inactivity can coincide with the presence or with the absence of corpora lutea, whilst the absence of any structural development, such as follicular growth, does not indicate endocrine rest. And it has also been shown that, in thallium-fed animals, the association between follicular growth and alpha production can be disrupted, the former appearing without the latter.

From a consideration of all these facts the following general conclusion emerges: *The nature of ovarian endocrine function is to be recognised in the responses which it provokes, and not by the ovarian structures with which it commonly coincides.*

These responses are discussed in the following paragraphs with a view to the particular conditions of experimentation.

4. The Identification of the Different Phases of Ovarian Activity by an Appeal to Responses evoked in the Genital Tract.

(A) *The Alpha Phase.*

The first phase of ovarian secretion is responsible for (*a*) the incitement of the mating reflex, (*b*) the enlargement of the uterus and the cornification of the vaginal epithelium after preceding proliferation, and (*c*) a rise in the rest metabolism which follows the stage of heat. All these effects appear to be incited by ovarian secretion during the alpha phase, and they occur in a definite order during this first phase of the sex cycle. Ordinarily, only one of these effects—cornification—is recorded, for the reason that it is the most easily identified. If all the

effects of the first phase were provoked by the same agent which induces cornification, then only this effect need be observed. It would seem probable, however, that there is at least one other factor involved, since it has not been possible to produce the typical rise in metabolism by injections of alpha hormone, and since it is still rather doubtful whether or not the mating reflex is induced by this hormone. Therefore, it is necessary to observe all these effects before the first phase of ovarian secretion can be regarded as being complete.

(B) *The Reproductive Phase.*

In order to establish the existence of the beta phase it is necessary to make use of certain characteristic changes in the uterus and in the vaginal epithelium, for this second phase of the sex cycle is remarkable in that (*a*) the uterus gains the potency to form decidual tissue, and (*b*) the epithelium of the vaginal wall becomes mucified. The pregnancy potencies of the uterus (*a*) can be demonstrated by drawing a fine thread through the uterus (Loeb). When the second phase of ovarian activity has been incited, the uterus reacts with the formation of deciduomata (Long and Evans, Evans and Simpson, Teel, Hisaw). It has also been shown that it is impossible to produce this reaction after injections of alpha hormone. The reaction has been produced, however, by Weichert (quoted after Hisaw), using extracts of corpus luteum of unstated preparation. Thus the occurrence of this reaction can certainly be regarded as an indication of the secretion of beta. On the other hand, the *absence* of the reaction is by no means a proof of the non-secretion of this hormone, for Hisaw has shown that extracts of corpus luteum do produce their characteristic effects, but only after the animal has already passed through an œstrous cycle. The placentation reaction, therefore, is more probably an effect of a combined, or rather successive, action of two hormones. Thus if the ovary secreted beta without first having elaborated alpha, no effect would be perceived by an observer who looked only for the placentation potency of the uterus.

Mucification (C), however, is a much more critical test. It is typical of the second phase of the genital cycle; it always accompanies true or pseudo-pregnancy; it can be produced by extracts of corpus luteum, and is invariably destroyed by the administration of alpha hormone in larger doses. Moreover, it is apparently independent of any preceding action of the alpha hormone, for Wiesner and Patel have produced mucification in the immature animal in which no œstrous cycle had yet occurred. It is suggested, therefore, that mucification is an adequate test of the existence of the second phase of ovarian secretion.

The existence of mucification cannot be recognised from an examination of the vaginal smear, for, as has been noticed by various authors—literature by Kelly (1928)—the vaginal smear during the interval is but very slightly different from that of pregnancy or pseudo-pregnancy—indeed, it is most difficult to distinguish between the smears of mucification and that of the stage of rest. Both contain nucleated epithelial cells and leucocytes. It follows that the vaginal smear cannot distinguish between ovarian inactivity and the second phase of ovarian secretion. In spite of the fact that the mucified vaginal epithelium differs very much in structure from the interval epithelium, to demonstrate the difference is possible only by the microscopical examination of sections of the vaginal wall.

(C) *Interval.*

It has been shown above that from an examination of the vaginal epithelium it is possible to identify the existence of the alpha and beta phases of the ovary; this examination can also reveal the existence of the phase of ovarian inactivity, for it has been shown that the absence of ovarian hormone is reflected in the vaginal wall. Double ovariectomy before puberty is followed by the persistence of the immature type of vaginal epithelium, whilst in the mature animal following ovariectomy the structure of the vaginal epithelium closely resembles that which it shows during the interval. Thus, absence of ovarian secretion leads to the persistence of low epithelium; on the other hand, injections of even sub-threshold amounts of alpha lead to a proliferation, but not to a cornification, of the epithelium; therefore the absence of such proliferation and the persistence of the immature type of epithelium may be regarded as an indication of the absence of any considerable amount of hormone in the system, and of the existence of a stage of ovarian inactivity.

The lack of a simple interdependency of ovarian structure and ovarian function is the more interesting, since in the normal cycle there is to be found a very definite co-ordination of morphological and physiological changes. As both are dependent on the anterior lobe, the problem which presents itself is how their co-ordination is related to the function of the latter. It has been shown that anterior lobe substance, or derivatives thereof, induce the formation of atretic bodies and structures (lutein cysts, follicular cysts, and hæmorrhagic follicles) which we are wont to regard as pathological. Therefore the problem of the structural changes produced by the anterior lobe becomes of particular interest in itself, but does not enter the discussion of the subject of this paper.

5. The Gonadotrope Action of Anterior Lobe Extracts.

Two problems thus present themselves. Neither phase of ovarian endocrine function occurs in the absence of the stimulus provided by the hypophysis. Does the pituitary incite the inception of either or both of these phases? Does there exist an interdependence of the two phases which normally occur in a certain sequence, the second being dependent on the stimulus—mating—which must be exerted during the first phase if it is to be effective?

Mice and rats were used as experimental animals. Since it was not possible to breed in this department all animals required, arrangements were made with the Mousery, Rayleigh, for weekly deliveries of mice which on the day of arrival, were four weeks of age. Before the experiment was started, and during its conduct, batches of these mice were tested for the first occurrence of œstrus. All experiments were separately controlled by a number of untreated animals of the same lot. The most frequent age of maturity in these stocks is considerably higher than that of the strain used by Engle and Rosasco, though the minimum age of maturity is almost the same. The early attainment of maturity in some individuals made it necessary to use a sufficient number of animals in each experiment in order to exclude errors statistically. Experimental animals and controls were fed on a diet of whole oats only. Many of the experimental animals were kept with young, though already mature, males, and were examined at least once, and, when possible, twice daily for vaginal plugs, since only the combined occurrence of mating and cornification indicates the normal first phase of ovarian secretion. As regards the second phase the method of Wiesner and Patel (excision of vaginal epithelium) for establishing mucification was used, whenever it was impossible to section the whole vagina. Care was taken to excise pieces both from the roof and the bottom of the vagina (dorsal and abdominal wall), and the excised pieces were always large enough to include parts of the proximal regions of the vagina. No loss occurred from these small operations, and after the slight bleeding from the wound had ceased, the animals did not require further attendance or care. Material for microscopic examination was fixed in Bouin's fluid freshly prepared.

The experiments with the implantation of pituitary substance were begun in 1927 and repeated in the summer and autumn of 1928. They comprised 43 mice and 11 rats, all of them immature females. Small pieces of anterior lobe were implanted in the manner described by Smith and Engle. Cattle pituitary was mostly used. One graft was implanted

to each animal in the muscle of the hind leg. The majority of animals (37) came in œstrus within one week after the graft had been administered. Comparisons as to the actual day when cornification occurred are of little value, because the size of the grafts varied. Six or seven grafted females, kept with males, mated. In all cases where cornification appeared it was found to be complete, in no way differing from that typical of the spontaneous cycle. Comparing the smears of these experimental animals with those of mature, untreated, normal mice, it was found that the duration of cornification was approximately the same in both cases. The accuracy of these observations is limited, since smears were taken only once daily.

These results are in agreement with those of previous workers (quoted above) as regards the œstrus-inducing effects of anterior lobe. We also can confirm the statement of previous authors that the implantation of pituitary grafts in ovariectomised animals is not followed by induction of the genital cycle. But, because of the phasic nature of ovarian secretion, we cannot conclude that the anterior lobe grafts induce "the" ovarian secretion, for all that has been shown so far is that they induce its first phase.

Thus it remained to be seen whether such grafts also induce, or can induce, the second phase. The first possibility to be tested was the automatic occurrence of the second phase as a necessary consequence of the first in the absence of any renewed pituitary stimulus. It has been pointed out that cornification represents a secondary effect of alpha hormone, the immediate effect of which is the proliferation of the vaginal epithelium. One could also assume that the production of beta automatically follows upon the production of alpha if the latter is not reincited again, thus interfering with the beta phase. But six animals which had received pituitary grafts at the age of twenty-eight days and had shown cornification were killed on the 4th, 5th, and 9th day respectively after cornification had disappeared, and the vaginæ of all these animals were in a state of rest. The uteri were of interval size. In only one case was slight proliferation, such as occurs in the initial stage of the œstrous cycle, found to prevail. The reason for this might have been that an œstrous cycle was just beginning, the animal having attained the age of spontaneous maturity. The other cases showed the interval type of epithelium, indicating the absence of any ovarian secretion, and thus there is no indication as to the occurrence of a beta phase subsequent to the alpha phase in any of these animals.

Thereupon we attempted to induce the beta phase by *repeated* grafts

of anterior lobe. Six immature animals twenty days old received grafts of anterior lobe every other day, and five implantations were made in each animal, but none showed any sign of a beta phase when killed twelve days after the first occurrence of cornification, and five or six days respectively after its disappearance. Some of these animals showed indications of an approaching œstrus when the vaginæ were cut, the epithelium being highly proliferated, partly stratified, and free of leucocytes. None of them showed mucification, even in its initial stages of development.

Thus it would appear that under the conditions prevailing in these experiments, grafts of anterior lobe induce only the alpha phase in the immature ovary. The effect of such grafts is *œstrogenic*. It cannot be stated, therefore, that the anterior lobe incites "the" ovarian secretion, but only that which is related to the first phase of the sex cycle.

The Preparation of Œstrogenic Extract.

In a number of implantation experiments it was found that anterior lobe fragments lose their efficacy when treated for some hours with alkali, strong mineral acids, or are boiled for half a minute. The œstrogenic substance is evidently highly sensitive to chemical interference. However, various methods of extracting œstrogenic substances from the anterior lobe have been elaborated. A very simple one is given below. In it sulphosalicylic acid is used. This acid was found to be particularly advantageous in these and other experiments with endocrine glands. It is a combined protein-precipitant, antiseptic, and extraction agent. Extracts which were subject to the action of this acid for some time were found to be sterile on being tested by the staff of the Royal College of Physicians Laboratory, to whom our thanks are due.

Example.—35 grms. anterior lobe of cattle pituitary are finely ground with sand, and 50 c.c. of 20 per cent. sulphosalicylic acid is added. The mixture is permitted to stand in a refrigerator from two to twelve hours. Then 60 c.c. of water is added. The mixture is shaken for half an hour, then filtered through a Buchner filter. The filtrate is passed through a membrane filter,* neutralised with sodium carbonate and injected. This extract, referred to in our records as No. 110, in daily doses of 0·1 to 0·2 c.c. proved to be œstrogenic.

Table I shows a representative series of experiments with this extract. It was administered to altogether 37 animals, samples of five different

* Obtained from the Membranfiltergesellschaft, Goettingen.

TABLES I. TO III.

	Animals.		Injections.			Smears. Days after first Injection.											Vagina. (Condition of Epithelium.)
	Record No.	Age at first Injection.	No. of	Amount. Daily.	Amount. Total.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	
		days															
I.	27.84	28	5	0·1	0·5	.	–	–	c	0	.	0	c	c	+	...	
	27.85	,,	5	0·4	2·0	.	–	–	0	c	.	0	.	.	+	...	
	27.86	,,	5	0·4	2·0	.	.	–	–	–	.	0	.	–	–	–	
	27.87	,,	5	0·8	4·0	.	.	–	–	–	.	0	–	–	–	...	
	27.100	,,	5	0·8	4·0	.	.	–	–	–	.	–	–	–	–	...	
	27.101	,,	4	0·8	3·2	.	.	–	–	–	.	c	–	–	–	–	
	27.102	,,	5	0·1	0·5	.	.	–	–	–	.	–	–	–	–	–	
	27.103	,,	5	0·1	0·5	.	.	–	–	+	...	...	...	...	...	...	
	27.106	,,	5	0·2	1·0	.	.	–	–	–	.	c	0	c	0	–	
	27.117	,,	5	0·2	1·0	.	.	–	–	–	.	–	–	–	–	...	
	27.118	,,	5	0·2	1·0	.	.	–	–	–	.	0	c	c	–	...	
	27.119	,,	5	0·2	1·0	.	.	–	–	–	.	+	...	...	...	...	
II.	25.2	42	3	0·2	0·6	.	.	.	–	–	–	–	–	–	+	...	Imm.
	25.3	,,	5	0·4	2·0	.	–	–	–	–	–	–	+	...	...	...	Imm.
	36.D.2	,,	5	0·2	1·0	.	.	.	–	–	–	–	+	...	...	...	Muc. II.
	36.D.4	,,	5	0·4	2.0	.	.	.	.	–	–	–	+	...	...	...	β-prol.
	38.C.6	35	4	0·4	1·6	–	–	–	–	–	.	.	+	...	...	...	Muc. II.
	38.C.7	,,	4	0·4	1·6	–	–	–	–	–	.	.	+	...	...	...	Muc. I.
	39.C.1	28	5	0·4	2·0	–	–	–	–	–	–	–	–	+	...	...	Muc. II.
	39.C.3	,,	5	0·4	2·0	–	–	–	–	–	–	–	–	+	...	...	Muc. I.
	39.C.4	,,	5	0·4	2·0	.	.	.	–	–	–	.	+	...	...	...	Muc. II.
	39.C.5	,,	5	0·4	2·0	.	.	.	–	–	–	–	+	...	...	..	Muc. III.
	43.B.5	,,	8	0·05	0·4	.	.	.	–	–	–	–	–	+	...	...	Muc. III.
	45.E.3	,,	3	0·4	1·2	.	.	–	–	–	.	+	...	...	...	...	Muc. II.
III.	70.D.1	35	3	0·2	0·6	.	.	+	...	..	...	...	...	...	...	...	Imm.
	70.D.2	,,	3	0·2	0·6	.	.	+	...	...	...	...	...	...	...	...	Imm.
	70.D.3	,,	3	0·2	0·6	.	.	+	...	...	...	...	...	...	...	...	Imm.
	70.D.4	,,	3	0·2	0·6	.	.	+	...	...	...	...	...	...	...	...	Imm.
	70.D.5	,,	3	0·2	0·6	.	.	–	+	...	..	...	...	...	..	...	β-prol.
	70.D.6	,,	3	0·2	0·6	.	.	–	+	...	...	...	...	...	...	...	β-prol.
	70.D.7	,,	3	0·2	0·6	.	.	–	–	+	...	...	...	...	...	...	Muc. I.
	70.E.4	,,	3	0·2	0·6	.	.	–	–	–	+	...	...	...	...	...	Muc. II.

. = No smear taken.
– = Smear containing nucleated cells and leucocytes.
c = Smear containing cornified cells and nucleated epithelial cells.
0 = Smear containing only cornified cells.
\+ = Killed.
Imm. = Immature.
β-prol. = Beta proliferation.

preparations being used. It will be noted that the duration of cornification in these experiments is decidedly shorter than in experiments with anterior lobe grafts, and the occurrence of cornification is shorter. Two out of five females which were kept with males mated.

In another series the vaginæ of some females which had not shown cornification after injection of the extract were sectioned; it was found that the vaginal epithelium persisted in the immature condition, or was slightly proliferated. Thus it would appear that this extract, under the conditions observed in these experiments, is merely œstrogenic where it is at all active.

The Preparation of Kyogenic Extracts.

Whereas no "beta-effect" was produced with the extract No. 110, alkaline extracts prepared according to the method used by Evans, Bellerby, and others were effective in inducing the beta-phase in immature ovaries. These extracts have been referred to, by the respective authors, as "œstrus-inhibiting." We can confirm that they do actually prevent the recurrence of œstrus in mature females for seven to twelve days, even though only 0·2 c.c. is given twice within the first three days of the experiment (*cf.* Bellerby).

The methods used in extraction deviated but little from that of Evans, Teel, and Bellerby.

Example.—65 grms. of anterior lobe (cattle pituitary) are finely ground with sand. 60 c.c. of N/10 sodium hydroxide are added. The mixture is allowed to stand overnight in the refrigerator at about freezing-point, or for about one hour at room temperature. Then it is neutralised with N/10 acetic acid, the neutralisation being carried on till "Universal Indicator B.D.H." reacts yellow-green, or phenol red reacts red. The extract is then centrifuged at high speed; the supernatant fluid is used for injection, and is referred to in our records as E. 119A.

Slight differences in the p^H (which according to Evans are of some importance for the content of such extracts of growth hormone) have but little influence upon the ability of the extracts to induce the second phase of ovarian function. The supernatant fluid collected through centrifuging has slightly toxic effects when given in amounts exceeding 0·4 c.c. daily to immature animals; but (as will be seen from Table II) much smaller amounts are already sufficient.

Altogether 47 animals were injected with E. 119A. In none of them did cornification occur, nor did any of the seven which were kept with males, mate. While these animals were mostly observed only for changes in the vaginal smear, a number of them were killed at intervals after the beginning of the injections and the vaginæ and the ovaries were sectioned.

The results of the microscopic examination are given in Table II. It was found that in only two out of twelve cases did the presence of leucocytes and nucleated epithelial cells in the vaginal smear correspond to an immature condition of the epithelium. In other cases the vagina was remarkable, sometimes even in its macroscopic appearance. It was large —compared with that of the immature animal—and showed folds. On sectioning, the wall was found to be thickened and lined with a mucified epithelium. The degree of mucification varied. Some animals showed an epithelium which did not differ in degree from that of mature pregnant females* (Muc. II) (*cf.* fig. 5, Plate I, and fig. 8, Plate II), whilst others even exceeded the degree of mucification reached by the pregnant animal (Muc. II) (*cf.* fig. 6, Plate II). In a number of animals the mucification, though not so pronounced, was well developed, whereas some showed early stages of this process (Muc. I and beta proliferation). Mucification was never observed in untreated immature animals, and occurs in the mature animal only after mating. The figures on Plates I and II show sections through the vaginæ of some of the treated animals.

On comparing mucification as it occurs in these animals with the vaginal wall in pseudo- or true pregnancy of normal mature animals, no qualitative difference could be detected, though, as has been stated, mucification in the experimental animal sometimes exceeds the highest stage ever observed in pseudo- or truly pregnant mature animals. Undoubtedly, in these animals the beta effect did occur.

Now, this effect cannot be due to the presence of beta hormone in the extracts themselves, for when ovariectomised animals were injected with the same amount, or even higher quantities, of the same extracts, no mucification was ever observed (fig. 2, Plate I).

Thus the effect of the extract No. 119A must be supposed to be an indirect one like that of pituitary grafts, or of No. 110. These alkaline extracts apparently have induced the beta phase in the immature way.

But whereas we can doubtless assume that the second ovarian phase actually has been induced by these extracts, it is still to be decided whether, in the immature female, it was induced directly and without the intermediate appearance of an alpha phase. It might well be that the extract at first induced alpha secretion, but that this was of short duration and/or low intensity, and was immediately followed and thereby obscured by beta secretion and mucification. If this were so, then the induction of the beta phase would not be direct. But no indication for such an alpha phase could be detected. When animals were treated with No. 119A and killed shortly after the first injection (2, 3, or 4 days respectively), it was found that in those which showed any effect at all, the vaginal epithelium was never

* Second half of pregnancy.

proliferated in such a way as it is under the influence of alpha in the early stages when the vaginal epithelium proliferates slightly into a stratified layer of cells, cubic or flat (*cf.* fig. 9, Plate II). On the other hand, in the early stages of the influence of No. 119A, the immature epithelium, though proliferated, exhibited an entirely different character (β-proliferation) (Table III). Its cells are higher than those formed under the influence of alpha, and no stratification appears (*cf.* fig. 7, Plate II). The structure of this epithelium closely resembles that of epithelium in the early stages of mucification as it develops after mating in the mature animal.

Thus there is no indication for the interpolation of an alpha phase between the immature condition of the epithelium and the attainment of mucification. On the other hand, there is every indication that the mucification develops directly from the immature condition of the epithelium. One must therefore conclude that a beta phase is induced in the immature ovary *directly* by the extracts, and without the previous appearance of an alpha phase, and that therefore the former appears to be independent of the latter.

In the normal mature animal the second phase occurs only after mating, which in its turn depends on the first phase of ovarian secretion. Thus the two phases are closely co-ordinated in the mature animal; but in spite of this fact the second phase neither follows automatically when the first phase has been induced, nor is it dependent for its occurrence on the previous appearance of the first phase. The two phases do not form a physiological unit: one can occur without the other, and their normal sequence does not represent the effect of an *Ablauf*, a series in which the latter phases must occur if and when the earlier phases have appeared, or are at least dependent on their previous occurrence.

The uterus of the animals in which complete or partial mucification had occurred differed but little from that of immature untreated controls. There was only a slight enlargement unaccompanied by any definite histological changes, and even this did not occur in all the animals. This might arouse comment. Moreover, since the occurrence of beta secretion is established in these animals, and since beta is the hormone which, in all probability, is responsible for the development of the potencies of the uterus significant of, and occurring in, pregnancy, one would expect such potencies to occur in the animals which showed mucification; deciduomata should be formed under any stimulus which in the pseudo-pregnant mature female incites their formation. It was found, however, that animals treated with an amount of No. 119A sufficient to produce complete mucification did not react to such a stimulus—drawing of threads

through the uterus—which in mature animals in the earlier stages of pseudo-pregnancy readily invokes the formation of placentomata. Whether the lack of response on the part of the uterus is due to the absence of a preceding alpha phase (as might be suggested in the light of Hisaw's experiments), whether the beta secretion was not sufficient, or whether any other cause is responsible for the failure to react, cannot now be decided. It seems, however, that the full effect of the beta hormone is not as unconditioned as is its production.

The non-production of deciduomata will require further investigation, but though significant in itself it does not reduce the certainty of the conclusion that in these experiments a beta phase has been produced. Thus it is established that two different gonadotrope actions can be exerted by the pituitary gland through substances contained in its anterior lobe. The two effects might appropriately be called *œstrogenic* and *kyogenic*. The first word needs no explanation. As regards the second, it will be remembered that beta is the hormone responsible for the occurrence of the pregnancy changes, both potential and actual, and any substance which induces its formation may rightly be called pregnancy-inducing or kyogenic.

It was found that œstrogenic extracts could produce both enlargement of the follicles and luteinisation of the theca and granulosa without previous rupture of the follicle, thus leading to the formation of atretic bodies; while kyogenic extracts also had enlarging influences upon the follicles exceeding a certain diameter proved to be atretic. Follicular cysts were observed in several animals; in others, lutein cysts occurred. These observations are in accordance with the findings of previous authors. The mechanism which leads to so widely differing results has been analysed in other experiments and will be discussed elsewhere, but there is one obvious result which needs to be mentioned in this context.

The ovaries of animals in which the injections did not lead to functional alteration of the immature ovary showed no morphological change. This applies both to the ovaries of animals injected with extract No. 110 and those of animals treated with extract No. 119A. On the other hand, the animals in which the first or second phase of ovarian secretion was incited showed ovarian structures varying greatly in different regions of the same ovary, and from animal to animal.

An interesting fact in this connection is that some of the animals in which cornification had occurred under the influence of extract No. 110 showed no definite follicular enlargement, no follicles with a diameter exceeding that of the follicles of the immature ovary being present.

There was no histological difference to be detected between the ovary of the immature control and the injected animal which had reacted with an alpha phase. It appears, therefore, that an amount of œstrogenic substance with suffices to induce alpha secretion does not lead to definite follicular enlargement.

As regards the kyogenic extracts, it was found that in some cases corpora lutea (or rather atretica) were formed. In others, changes in the immature ovary were restricted to a thickening of the theca and a certain transformation of the cells, both of the peripheric layers of the granulosa and apparently also the theca. The cells thus formed are larger than those of granulosa and resemble lutein cells, though not always equalling them in size. It must be noted that animals in which the changes in the ovary were restricted to the formation of these luteoid peripheric follicular zones were, nevertheless, beta-active. No true and normal lutein tissue seems to be required for the formation of beta, although this would seem to be the physiologically active agent that has been referred to as the corpus luteum hormone.

Thus it appears that these extracts induce production of the respective hormones without provoking the structural changes which normally precede, accompany, or follow their elaboration.

Since it has been shown in those experiments that the two phases of ovarian action can be induced independently of each other, and that pituitary extracts can induce either phase, it becomes inadequate to state that the anterior pituitary induces *maturation* of the immature ovary. It can induce either of the phases of maturity, and the problem to be decided in every case is which phase actually has been induced. Of necessity the statement must be made that tests thus far used for pituitary extracts and other substances are inadequate, or at least incomplete.

For changes in the ovary such as appear during the œstrous cycle prove the presence of pituitary hormone in immature animals, since such changes never occur in the hypophysectomised animal. But their absence, or the absence of the formation of corpora lutea and the non-appearance of cornification, does not indicate the absence of pituitary hormone, or even its presence in sub-threshold amounts. For it is obvious that the absence of both cornification and structural changes, such as follicular enlargement or thickening of the theca, may be combined with a high activity induced by the kyogenic substance of the pituitary. The only tests for the activating influence of the pituitary upon ovarian secretion which are qualitatively sufficient are the changes in the vaginal epithelium. This statement, initially deduced from considerations of the physiology of the sex cycle in its

normal and abnormal conditions, is amply borne out by the results of these experiments.

The kyogenic effects of alkaline extracts might have been expected from an adequate interpretation of Evans' fundamental work. For this author, and others of his school, report that after prolonged treatment of female rats with these extracts, the uteri of the animals react to stimulation by forming deciduomata. Since then our knowledge has developed, and we now know that this formation is an effect of the second phase of ovarian secretion.

6. The Nature of the Sex Hormone of the Anterior Pituitary.

In consideration of the kyogenic action of the anterior lobe, it will be noted that certain conclusions of Evans and Simpson require further analysis. It has been mentioned above that, according to Smith and Engle, the œstrogenic effect of pituitary grafts is inhibited by pituitary extracts prepared with alkali. In repeating these experiments we could confirm that actually no œstrus occurs if alkaline extracts are injected before, or together with, the graft; but in these cases the animals showed strong mucification, and thus the contrast between the two actions is not one of suppression of the sex hormone, not one of activation and inactivation of the immature gonad, but is a contrast of two activations of the gonad, qualitatively different. It appears that the kyogenic extract proves to be stronger in these experiments than the œstrogenic graft, but both extract and graft could rightly be assumed to contain a "sex hormone" of the anterior lobe—or, more generally expressed, a gonadotrope substance. This latter expression seems to us more appropriate, for while the superordinated position of the pituitary in the field of ovarian function is placed beyond doubt by the fundamental researches of Evans, Smith and Engle, Zondek and Aschheim, obviously the facts are too complicated to warrant as simple a conclusion as that put forward by those authors who assume the existence of "a" sex hormone. It has been pointed out before that these authors consider the œstrous cycle to be not only the foremost, but even the only significant effect of ovarian action. So long as only one such action was recognised, so long as the œstrous cycle in the mind of investigators represented the whole complex of ovarian activity, and so long as the only known action of the pituitary upon gonadic function was the induction of œstrous activity, it was possible to think of a superordinated sex hormone. But since we are dealing with different phenomena occurring in the gonad under the influence of pituitary substances, and thus it becomes necessary to ask why these substances activate the ovary in two different ways and

to two different ends; why some extracts are œstro- and others kyo-genic. One has to consider the existence of two different effects, and first to find out whether they are due to quantitative differences in the amount of a sex hormone present in the extracts, or to qualitative differences in its constitution, indicating the existence of more than one gonadotrope substance in the anterior lobe.

Though not finally proving the existence of more than one sex-activating substance, experiments of ours indicate that one has to assume a qualitative difference of the kyogenic and œstrogenic extracts rather than a quantitative difference only. Assuming that there exists one substance, one sex hormone, the "x" previously suggested, then one of two relations can prevail: (*a*) a small amount of "x" is œstrogenic, a large amount is kyogenic; (*b*) a large amount of "x" is œstrogenic, and a small amount is kyogenic.

We tested first the assumption (*a*). If (*a*) is correct, then a reduction in the amount of kyogenic extracts which produces mucification should eventually reach a point where this kyogenic extract becomes œstrogenic. But this is not the case, as has been found in a number of experiments where the amount of No. 119A daily for five days injected in animals varied between 0·0012 c.c. (used in proper dilution of the original extract) and 0·2. The results found varied in as much as the majority of the animals receiving 0·0025 c.c. or more showed mucification of varying degree, whereas the animals injected with smaller amounts were found to have a vaginal epithelium hardly exceeding the stage of immaturity. In two cases the vaginæ showed slight proliferation of the kind which precedes mucification, but in no case did cornification appear. These experiments comprised 15 animals, and were conducted with one and the same sample of No. 119A. This experiment indicates that the assumption (*a*) does not correspond to facts.

As regards assumption (*b*), one has to conclude that repetition of the administration of kyogenic extracts, or an increase in their amount, would lead to an œstrogenetic effect—in other words, that a greater amount of No. 119A than that producing beta secretion would lead to alpha secretion. To prove this we injected large amounts of No. 119A up to 0·5 c.c. daily for five days. Since 0·025 c.c. of No. 119A injected three times can already be kyogenic, this injection induces probably a hundred times the kyogenic minimum.* But all these animals showed mucification of quite enormous

* It has been usual in work in ovarian hormones and pituitary hormones to speak of units. The standardisation of extracts is generally very difficult, and it seems that the difficulties in standardising ovarian hormones are even exceeded in work with pituitary extracts. When we speak of a minimum we refer to observed minima without trying to create anything like a unit, or even finally defining its physiological standards.

intensity, and thus the experiment did not support assumption (*b*). Moreover, 0·5 c.c. of this extract represents about 0·4 grm. of anterior lobe substance, whereas a much smaller amount when implanted suffices to induce œstrus.

Thus it follows that there is no basis for the assumption that the kyogenic and œstrogenic actions of the extracts are due to simply quantitative differences in their content of one and the same substance.

There is evidence, on the other hand, which seems to show that actually two substances of pituitary origin and possessing distinctive physiological properties are involved. If one assumes with Evans that there are a sex hormone and also a growth hormone, then one might be tempted to adopt the simplest possible hypothesis and to assume that the sex hormone alone is œstrogenic, whereas, in company with the growth hormone, it is kyogenic. This assumption can be tested since it is possible to free extracts such as No. 119A of the growth factor by appropriate methods.

Example.—A quantity of No. 119A is rendered protein-free by the addition of 20 per cent. sulphosalicylic acid, the necessary amount of which must be determined from case to case and then filtered. The filtrate is then neutralised with sodium carbonate (B.D.H. indicator pea-green).

No growth effect of this extract could be noted while it induced mucification. Thus it appears that the association of the growth effect and the kyogenic effect can be disrupted.

It is reasonable, therefore, to postulate the existence of two gonadotrope factors as well as a growth factor of the anterior lobe. In order to facilitate the discussion and to construct a useful working hypothesis, we shall refer to these two gonadotrope factors as "Rho 1" and "Rho 2" respectively. Such names suggest the insecurity and the transient nature of the argument.

It is assumed that Rho 1 alone, or, in a combination of Rho 1 and Rho 2, a quantitative superiority of Rho 1, is responsible for the incitement of the first phase of ovarian activity. Under the impact of Rho 1 (the œstrogenic agent) the ovary proceeds to the elaboration of alpha: Rho 2, or a preponderance of it, is responsible for the incitement of the second ovarian phase, Rho 2 thus being the kyogenic factor inciting the elaboration of the beta factor by the ovary.

A survey of the literature on the physiology of the sex cycle will show that there is but little in it which cannot be accommodated, or even more properly explained, by this hypothesis than by any previously suggested. It removed, for example, those difficulties inherent in the conception of the physiologically significant differences between follicular and luteal phases of ovarian activity. It has been shown that these cannot

be regarded as physiological realities. This being so, a more helpful hypothesis, such as that which is adumbrated in this paper, becomes necessary.

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